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(54) Title: USE OF 5-ALPHA-REDUCTASE INHIBITORS TO INCREASE HDL CHOLESTEROL LEVELS

(57) Abstract

The present invention relates to the use of 5α-reductase inhibitors to increase serum HDL cholesterol levels. These compounds may be used together with other lipid lowering agents, such as HMG-CoA reductase inhibitors, squalene synthase inhibitors, HMG-CoA synthase inhibitors, bile acid sequestrants, niacin, probucol, and the fibric acids to reduce the risk of coronary artery disease mortality. Further, two or more 5α-reductase inhibitors may be used in combination to increase serum HDL cholesterol levels.

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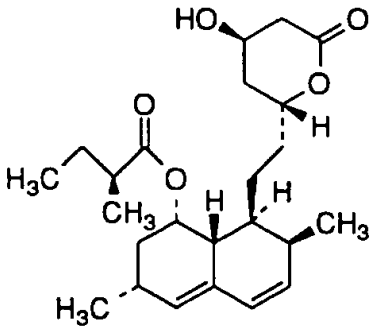
TITLE OF THE INVENTION

USE OF 5-ALPHA-REDUCTASE INHIBITORS TO INCREASE HDL CHOLESTEROL LEVELS

BACKGROUND OF THE INVENTION

Cholesterol is a major building block of all plasma membranes and a key compound for the synthesis of steroid hormones and bile salts. Cholesterol and triglycerides are lipophilic and cannot dissolve in water and thus cannot travel through the bloodstream in their unaltered forms. They are made water-soluble by combining with proteins produced by the liver and intestine called apolipoproteins and the complexes thus formed are called lipoproteins, which vary in size, weight and density. Two of the major classes are called low-density lipoproteins (LDLs) and high density lipoproteins (HDLs). LDL contains 60 to 70 percent of serum cholesterol and is believed to pick up cholesterol and deposit it in body cells, including smooth muscle cells in arteries. As blood levels of LDL increase, the risk of coronary artery disease increases. HDL, which contains 20 to 30 percent of total serum cholesterol, is believed to gather cholesterol from body cells and transport it to the liver for elimination. As blood levels of HDL increase, the risk of coronary artery disease decreases. A third type of lipoprotein, known as very low density lipoprotein (VLDL) contains about 10 to 15 percent of total serum cholesterol and a large amount of fat. Thus, to reduce the risk of coronary artery disease, the overall goal has been to increase HDL cholesterol levels and to lower LDL and VLDL cholesterol levels.

MEVACOR® (lovastatin), ZOCOR® (simvastatin), PRAVACHOL® (pravastatin) and LESCOL® (fluvastatin), now commercially available, are members of a group of very active antihypercholesterolemic agents that function by lowering cholesterol biosynthesis by inhibiting the enzyme HMG-CoA reductase. These compounds are effective in lowering LDL and VLDL cholesterol levels, but do not exhibit a large effect in increasing HDL levels.



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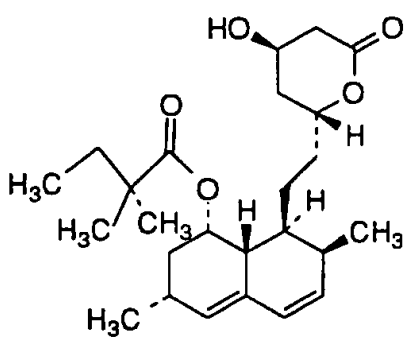

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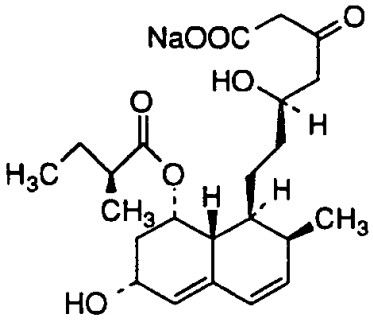

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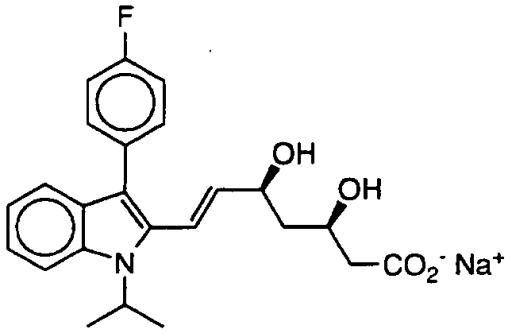

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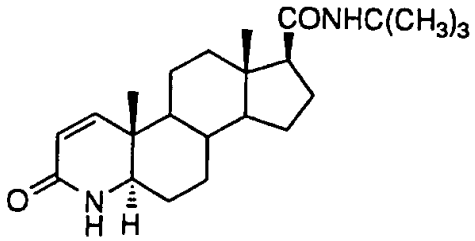
Current methods for increasing HDL cholesterol include exercise, moderate alcohol consumption and several lipid-lowering drugs, including niacin (nicotinic acid), fibrates such as gemfibrozil, and inhibitors of HMG-CoA reductase, such as lovastatin and simvastatin. However, exercise and alcohol are not acceptable to many patients. Patient compliance is difficult with exercise, and many physicians do not feel comfortable encouraging alcohol consumption, which in excess can lead to problems such as cirrhosis of the liver. Each of the lipid-lowering drugs have particular side effects. Niacin, which produces the largest increases in HDL cholesterol, also has the most annoying and clinically significant side effects, including flushing in almost all patients, dyspepsia and hepatotoxicity, which greatly reduce patient compliance. The fibrates have the side effects of gastrointestinal disturbances and an increased risk of gallstones.

Although HMG-CoA reductase inhibitors are relatively safe and well-tolerated, their effect on HDL-cholesterol is small, usually about less than 10%, even at maximum dose.

The compounds employed in the present invention are 5α-reductase inhibitors. Finasteride (PROSCAR®) is a commercially available 5α-reductase inhibitor,



Finasteride

currently approved for the treatment of benign prostatic hyperplasia (BPH). Inhibition of 5α-reductase is extremely well-tolerated, probably because the only known function of the enzyme in adults is to support certain male secondary sexual characteristics, including prostate growth. Males who are homozygous for 5α-reductase deficiency have small prostates and do not develop BPH. Women who are homozygous for 5α-reductase deficiency are phenotypically normal, indicating that the enzyme serves no general biological purpose. Finasteride, the first member of this class to be available for prescription, is a very well-tolerated drug.

It is well known that the concentration of HDL cholesterol is lower in men than in women. This is true for post-menopausal as well as pre-menopausal women, suggesting that it is the high level of androgens, and not the low levels of estrogens, that depresses HDL cholesterol in men. When boys enter puberty , HDL cholesterol falls, adding further support to this concept. The two principal androgens are testosterone and dihydrotestosterone; the latter of which is formed from testosterone by the action of the enzyme 5α-reductase. It is not known

which of these androgens is the more important in the control of HDL cholesterol levels. Until the present invention, it was not known whether dihydrotestosterone contributes to the process of depressing HDL cholesterol levels and whether inhibitors of 5α-reductase might increase the levels of HDL cholesterol.

SUMMARY OF THE INVENTION

Administration of 5α-reductase inhibitors increases serum HDL cholesterol levels. These compounds may be used alone or together with lipid lowering agents, such as HMG-CoA reductase inhibitors, squalene synthase inhibitors, HMG-CoA synthase inhibitors, bile acid sequestrants, niacin, probucol, and the fibric acids to reduce the risk of coronary artery disease mortality and morbidity. Further, two or more 5α-reductase inhibitors may be used in combination to increase serum HDL cholesterol levels.

DESCRIPTION OF THE INVENTION

This invention relates to the use of 5α-reductase inhibitors to increase serum (or plasma) HDL cholesterol levels either alone or in combination with other lipid-altering agents.

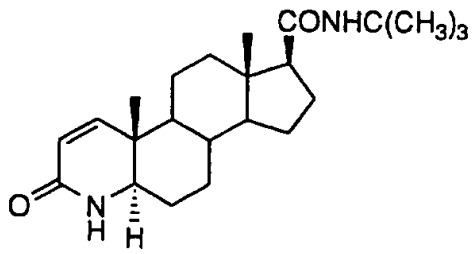
The term "5α-reductase inhibitor" as used herein is intended to include compounds which are active as inhibitors of either or both of the isozymes of 5α-reductase, such as, e.g., inhibitors of 5α-reductase type 1, such as, e.g., 4,7β-dimethyl-4-aza-5α-cholestan-3-one (also known as MK-386; as disclosed in WO 93/23420 to Merck & Co., Inc.), inhibitors of 5α-reductase type 2, such as, e.g., finasteride, epristeride (also known as SKF-105657, SmithKline Beecham), ONO-3805 (Ono Pharmaceutical Co., Ltd.), FK-143 (Fujisawa), KF-18678 to Kyowa Hakko-Koygo and TZP-4238 (Teikokuzoki), and those which are active as dual inhibitors of both isozymes type 1 and 2, such as, e.g., those disclosed in WO 94/00121 and WO94/00125 to SmithKline Beecham and WO 93/13124 and WO 94/14833, including epimasticadienolic acid to Glaxo. Also encompassed by the instant method invention is the use of a combination of an inhibitor of 5α-

reductase type 1 with an inhibitor of 5α-reductase type 2, such as, e.g., the use of a combination of finasteride with MK-386. Many compounds which are 5α-reductase inhibitors have been described in the art; compounds which are 5α-reductase inhibitors can also be determined by the 5α-reductase assay, further described below.

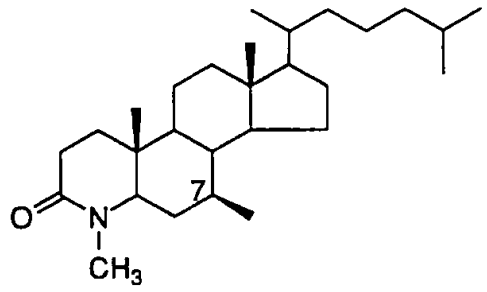
Examples of compounds which are 5α-reductase inhibitors include, but are not limited to, those described in the following patents and publications: U.S. Patent No's: 5,017,568; 5,061,803; 5,061,801; 5,061,802; 5,026,882; 4,377,584; 4,760,071; 4,845,104; 4,859,681; 5,049,562; 5,120,742; 5,138,063; 5,302,528; 5,302,528; and 5,151,429; and WO 93/13124; WO 93/23038; WO 93/23039; WO 93/23040; WO 93/23041; WO 93/23048; WO 93/23050; WO 93/23051; WO 93/23419; WO 93/23420; WO 93/16996; WO 93/23042; WO 93/24442; WO 94/00121; WO 93/25568; WO 91/13060; WO 94/00125; WO 94/03474; WO 94/03475; WO 94/03476; WO 94/07909; WO 94/11385; WO 93/13124; WO 94/13691; WO 94/14833; EP 532,190; EP O 511 477; EP O 484 094; EP O 453 321; EP O 294 937; EP O 291 245; JP O 5 331 059; FR 2,698,791; and FR 2,693,461. Compounds within these publications are included as compounds which may be used in the method of the present invention. The above list is not intended to be exhaustive, and there are many other publications which describe inhibitors of 5α-reductase.

Especially preferred 5α–reductase inhibitors are the following:

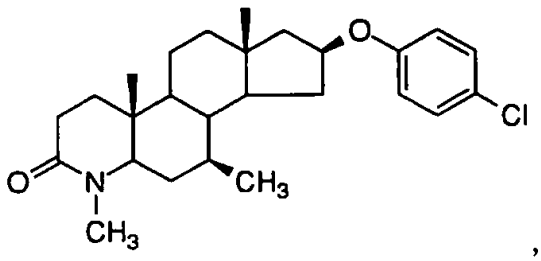
(1) finasteride (PROSCAR®)



(2) 4,7β-dimethyl-4-aza-5α cholestan-3-one

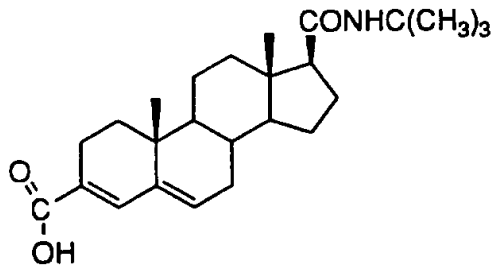


(3) 3-oxo-4-aza-4,7-β-dimethyl-16β-(4-chlorophenoxy)-5α-androstane



, and

(4) epristeride



and pharmaceutically acceptable salts thereof.

The 5α-reductase inhibitors of the present invention may also be beneficially administered as a combination of 5α-reductase inhibitors. The term "administration" refers to both concurrent and sequential administration of the active agents. Especially preferred is a combination of a Type 1 and a Type 2 5α-reductase inhibitor.

Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts include the following salts:

Acetate	Lactobionate
Benzenesulfonate	Laurate
Benzoate	Malate
Bicarbonate	Maleate
Bisulfate	Mandelate
Bitartrate	Mesylate
Borate	Methylbromide
Bromide	Methylnitrate
Calcium Edetate	Methylsulfate
Camsylate	Mucate
Carbonate	Napsylate
Chloride	Nitrate
Clavulanate	N-methylglucamine
Citrate	ammonium salt
Dihydrochloride	Oleate
Edetate	Oxalate
Edisylate	Pamoate (Embonate)
Estolate	Palmitate
Esylate	Pantothenate
Fumarate	Phosphate/diphosphate
Gluceptate	Polygalacturonate
Gluconate	Salicylate
Glutamate	Stearate
Glycollylarsanilate	Sulfate
Hexylresorcinate	Subacetate
Hydrabamine	Succinate
Hydrobromide	Tannate
Hydrochloride	Tartrate

Hydroxynaphthoate	Teoclate
Iodide	Tosylate
Isothionate	Triethiodide
Lactate	Valerate

The term "pharmacologically effective amount" shall mean that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by a researcher or clinician.

The term "alkyl" shall mean straight or branched chain alkanes of one to ten total carbon atoms, or any particularly specified number of carbon atoms.

Whenever the term "alkyl" or its prefix root appears in a name of a substituent (e.g., aralkoxyaryloxy) it shall be interpreted as including those limitations given above for "alkyl", unless otherwise indicated.

The compounds of the present invention can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixers, tinctures, suspensions, syrups and emulsions. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount of the compound desired can be employed as an agent to increase HDL cholesterol levels.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

Oral dosages of the present invention, when used for the indicated effects, will range between about 0.05 to 1000 mg/day orally. The compositions are preferably provided in the form of tablets containing 0.2, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0 and 50.0 mg of active ingredient. Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, preferred compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittant throughout the dosage regimen. Other preferred topical preparations include creams, ointments, lotions, aerosol sprays and gels, wherein the concentration of active ingredient would range from 0.1% to 15%, w/w or w/v.

In the methods of the present invention, the compounds herein described in detail can form the active ingredient, and are typically administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium

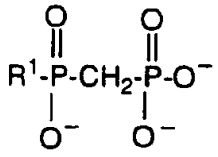
oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.

The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine or phosphatidylcholines.

Compounds of the present invention may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamidephenol, polyhydroxy-ethylaspartamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, *polyepsilon* caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

The compounds of this invention may also be administered in combination with other cholesterol lowering agents such as those which inhibit an enzymatic pathway in the biosynthesis of cholesterol. Such administration includes concurrent as well as sequential administration. Examples of such agents would include but are not limited to HMG-CoA reductase inhibitors, HMG-CoA synthase inhibitors, squalene epoxidase inhibitors and squalene synthase inhibition. Illustrative of such HMG-CoA reductase inhibitors are lovastatin and related compounds as disclosed in U.S. Patent No. 4,231,938 simvastatin and related compounds such as disclosed in U.S. Patent No. 4,450,171 and 4,346,227 pravastatin and related compounds such as disclosed in U.S. Patent No. 4,346,227

and fluvastatin and related compounds such as disclosed in WO 84/02131. Examples of HMG-CoA synthase inhibitors are: the beta-lactone derivatives disclosed in U.S. Patent No. 4,806,564, 4,816,477, 4,847,271, and 4,751,237; the beta lactam derivatives disclosed in U.S. 4,983,597 and the substituted oxacyclopropane analogues disclosed in European Patent Publication EP O 411 703. The squalene synthetase inhibitors suitable for use herein include, but are not limited to, those disclosed by Biller *et al., J. Med. Chem.*, 1988 Vol. 31, No. 10, pp. 1869-1871, including isoprenoid (phosphinylmethyl)-phosphonates such as those of the formula



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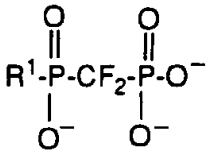

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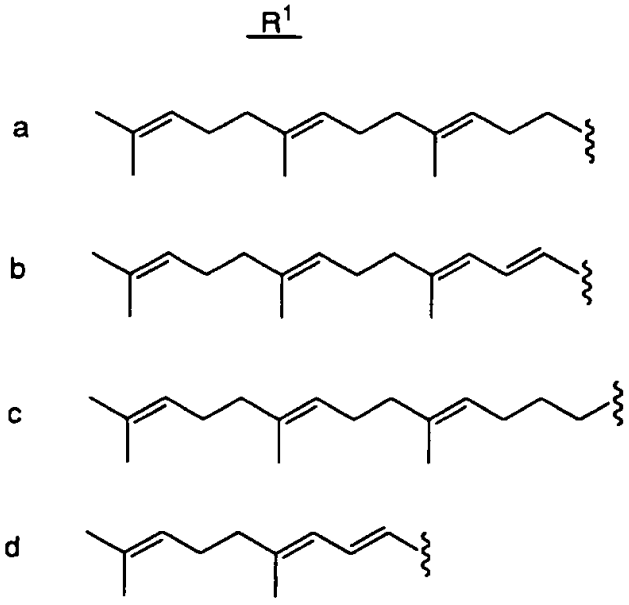


including the triacids thereof, triesters thereof and tripotassium and trisodium salts thereof as well as other squalene synthetase inhibitors disclosed in pending U.S. Patent No. 4,871,721 and 4,924,024 and in Biller *et al., J. Med.Chem.*, 1988, Vol. 31, No. 10, pp. 1869 to 1871.

In addition, other squalene synthetase inhibitors suitable for use herein include the terpenoid pyrophosphates disclosed by P. Ortiz de Montellano *et al., J. Med. Chem.*, 1977, 20, 243-249, the farnesyl diphosphate analog A and presqualene pyrophosphate (PSQ-PP) analogs as disclosed by Corey and Volante, *J. Am. Chem. Soc.* 1976, 98, 1291-1293, phosphinylphosphonate reported by McClard, R. W. *et al., J.A.C.S.*, 1987, 109, 5544 and cyclopropanes reported by Capson, T.L., PhD dissertation, June, 1987, *Dept. Med. Chem. U. of Utah*, Abstract, Table of Contents, pp. 16, 17, 40-43, 48-51, Summary.

Further, the benzodiazepine squalene synthase inhibitors described in EP O 567 026 to Takeda Chemical Industries, the quinuclidinyl squalene synthase inhibitors described in PCT publications WO 94/03451, WO 93/09115, WO 93/21183, WO 93/21184, WO 93/24486, and U.S. 5,135,935, may be employed in combination with the 5α-reductase inhibitors of the present invention. In addition, the zaragozic acid type squalene synthase inhibitors as described in U.S. Patents 5,284,758; 5,283,256; 5,262,435; 5,260,332; 5,264,593; 5,260,215; 5,258,401; 5,254,727; 5,256,689; 5,132,320; 5,278,067, and PCT Publications WO 92/12156; WO 92/12157; WO 92/12158; WO 92/12159; WO 92/12160; WO 93/18040; WO 93/18039; WO 93/07151; and European Patent Publications EP O 512 865, EP O 568 946; EP O 524,677 and EP O 450 812, as well as the acyclic tricarboxylic acid compounds 5,254,727, may be employed. Illustrative examples of squalene epoxidase inhibitors are disclosed in European Patent Publication EP O 318 860 and in Japanese Patent Publication JO2 169-571A. LDL-receptor gene inducer molecules are disclosed in U.S. Patent Application Serial No. 07/670,640 filed March 18, 1991.

Other cholesterol lowering agents that may be administered include niacin, probucol, and the fibric acids, clofibrate and gemfibrozil.

The dose of HMG-CoA reductase inhibitor contemplated for use in the co-administration of the present invention are from about 1 to 200 mg per day, preferably given in single or divided doses. Most preferred are dosages from 5 to 80 mg per day.

The doses of HMG-CoA synthase inhibitor contemplated for use in the co-administration of the present invention are from about 20 to 200 mg, preferably given in single or divided doses.

The doses of squalene synthase inhibitor contemplated for use in the co-administration of the present invention are from about 2 to 2000 mg per day, preferably given in single or divided doses.

The doses of squalene epoxidase inhibitor contemplated for use in the co-administration of the present invention are from 2 to 200 mg per day, preferably given in single or divided doses.

Representative of additional combinations are those containing 0.01 to 1000 mg of a 5α-reductase inhibitor in combination with up to 1000 mg probucol, up to 2 g clofibrate, 0.5 to 8 g of niacin, 800 to 1500 mg gemfibrozil or fenofibrate, or 20 to 300 mg of an LDL receptor gene inducer.

The 5α-reductase inhibitors of the present invention may also be co-administered with pharmaceutically acceptable nontoxic cationic polymers capable of binding beta acids in a non-resorbable form in the gastrointestinal tract. Examples of such polymers include cholestyramine, colestipol, and poly[methyl-(3-trimethylaminopropyl)imino-trimethylene dihalide].

The relative amounts of the compounds of this invention and these polymers is between 1:100 and 1:15,000.

Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these compounds.

EXAMPLE 1

Analysis of the Effect of Finasteride, a 5α-reductase Inhibitor, on Lipids

Study Design

A 60-week, double-blind, radomized, placebo-controlled, multicenter study was conducted in which 137 healthy male subjects were assigned to begin the 2 week placebo run-in period at the Week -2 visit. The subjects were allocated to receive either finasteride 5 mg (PROSCAR®) or placebo for 24 weeks at the Week 1 visit.

At the end of the active treatment period (Week 24) subjects discontinued the assigned medication. Thirty-six weeks after stopping the medication (Week 60), subjects were seen in the clinic for the final visit.

All medication was taken once a day in the morning before breakfast. All subjects were instructed to take their medication one hour before their scheduled clinic visit.

Fasting lipid profile (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, Lp(a), apoAI, AII, B) was obtained at Weeks 1, 12, 24 and 60. The subject must have fasted for a minimum of 12 hours. The serum was frozen at -20°C for shipment to a central reference laboratory for analysis.

Other than fasting before the office visit, subjects consumed a normal diet throughout the study.

Results

Subjects who had not been fasting were excluded from the analysis. Tables 1 and 2 contain the results of the percent change at Week 12 or Week 24, respectively, from Week 1 (baseline) for total cholesterol (tot. chol), HDL cholesterol (hdl), LDL cholesterol (ldl), and triglycerides (trig).

In the Tables below, "N" represents the number of subjects in the group, "mean" is the mean, "std" is the standard deviation, and "med" is the median value.

TABLE 1

Week 12: Percent change from Week 1

variable	group	N	mean	std	med	p-value within	p-value between
tot. chol							
	PLACEBO	51	1.6	9.8	2.8	0.211	
	PROSCAR®	56	1.5	13.5	0.7	0.449	0.994
hdl							
	PLACEBO	51	-0.2	12.1	0.0	0.784	
	PROSCAR®	56	4.5	14.7	2.1	0.019	0.081
ldl							
	PLACEBO	51	2.0	14.0	2.5	0.454	
	PROSCAR®	56	0.6	19.2	1.6	0.878	0.681

TABLE 2

Week 24: Percent change from Week 1

variable	group	N	mean	std	med	p-value within	p-value between
tot. chol							
	PLACEBO	48	0.4	12.1	0.2	0.755	
	PROSCAR®	53	4.7	11.8	3.2	0.003	0.072
hdl							
	PLACEBO	48	2.8	12.4	2.1	0.213	
	PROSCAR®	53	9.1	14.5	6.8	0.000	0.022
ldl							
	PLACEBO	48	-3.3	16.7	-4.3	0.172	
	PROSCAR®	53	2.6	18.5	0.9	0.565	0.100

Tables 3 and 4 contain the results at Week 12 and Week 24, respectively, of change from Week 1 in mg/dl for each variable, i.e., the change in each measurement unit for each of the above variables.

TABLE 3

Week 12: Change from Week 1 in mg/dl

variable	group	N	mean	std	med	p-value within	p-value between
tot. chol							
	PLACEBO	51	2.0	18.9	5.0	0.377	
	PROSCAR®	56	1.4	23.8	1.0	0.580	0.882
hdl							
	PLACEBO	51	-0.5	5.6	0.0	0.609	
	PROSCAR®	56	1.5	7.3	1.0	0.043	0.134
ldl							
	PLACEBO	51	0.5	15.6	2.0	0.686	
	PROSCAR®	56	-1.0	19.4	2.0	0.939	0.649

TABLE 4

Week 24: Change from Week 1 in mg/dl

variable	group	N	mean	std	med	p-value within	p-value between
tot. chol							
	PLACEBO	48	-0.9	24.6	0.5	0.988	
	PROSCAR®	53	7.0	20.5	7.0	0.005	0.084
hdl							
	PLACEBO	48	1.0	5.8	1.0	0.432	
	PROSCAR®	53	3.8	6.4	3.0	0.000	0.023
ldl							
	PLACEBO	48	-5.1	21.2	-4.0	0.099	
	PROSCAR®	53	1.6	18.4	1.0	0.590	0.090

HDL

There was a significant HDL percent increase (mean = 4.5%) from Week 1 to Week 12 for the finasteride 5 mg (PROSCAR®) treatment group. From Week 1 to Week 24, the increase doubled (mean = 9.1%) for the finasteride group.

For the placebo group, there was minimal HDL percent change (mean = -0.2% from Week 1 to Week 12). The mean of the HDL percent change was 2.8% from Week 1 to Week 24 for the placebo group. This 2.8% increase was not statistically significant.

Between the two treatment groups, the difference of 4.7% in the mean of the HDL percent change from Week 1 to Week 12 was not significant ($p = 0.081$). The difference of 6.3% in the mean of the HDL percent increase from Week 1 to Week 24 was significant ($p = 0.022$).

The absolute change in HDL increased 3.8 mg/dl from Week 1 to Week 24 for the finasteride 5 mg group and 1.0 mg/dl for the placebo group. The 2.8 mg/dl absolute difference between the two treatment groups was statistically significant.

LDL

There was no significant change in LDL for either group. The group treated with finasteride had an increase in LDL of 2.6% (1.6 mg/dl) from Week 1 to Week 24. The placebo group had a decrease in LDL of 3.3% (5.1 gm/dl) from Week 1 to Week 24.

Total Cholesterol

There was a significant total increase (4.7% or 7.0 mg/dl) from Week 1 to Week 24 for the finasteride group. There was no significant change for the placebo group. The increase in total cholesterol for the finasteride group was not statistically significant relative to the placebo group.

EXAMPLE 2

BIOLOGICAL ASSAYS

Preparation of Human prostatic and scalp 5α-reductases

Samples of human tissue were pulverized using a freezer mill and homogenized in 40 mM potassium phosphate, pH 6.5, 5 mM magnesium sulfate, 25 mM potassium chloride, 1 mM phenylmethyl-sulfonyl fluoride, 1 mM dithiothreitol (DTT) containing 0.25 M sucrose using a Potter-Elvehjem homogenizer. A crude nuclear pellet was prepared by centrifugation of the homogenate at 1,500 x g for 15 min. The crude nuclear pellet was washed two times and resuspended in two volumes of buffer. Glycerol was added to the resuspended pellet to a final concentration of 20%. The enzyme suspension was frozen in aliquots at -80°C. The prostatic and scalp reductases were stable for at least 4 months when stored under these conditions.

5α-reductase assay

The reaction mixture for the type 1 5α-reductase contained 40 mM potassium phosphate, pH 6.5, 5 mM [7-^{3}H]-testosterone, 1 mM dithiothreitol and 500 μM NADPH in a final volume of 100 μl. The reaction mixture for the type 2 5α-reductase contained 40 mM sodium citrate, pH 5.5, 0.3 mM [7-^{3}H]-testosterone, 1 mM dithiothreitol and 500 μM NADPH in a final volume of 100 μl. Typically, the assay was

initiated by the addition of 50-100 µg prostatic homogenate or 75-200 µg scalp homogenate and incubated at 37°C. After 10-50 min. the reaction was quenched by extraction with 250 µl of a mixture of 70% cyclohexane: 30% ethyl acetate containing 10 µg each DHT and T. The aqueous and organic layers were separated by centrifugation at 14,000 rpm in an Eppendorf microfuge. The organic layer was subjected to normal phase HPLC (10 cm Whatman partisil 5 silica column equilibrated in 1 ml/min 70% cyclohexane: 30% ethyl acetate; retention times: DHT, 6.8-7.2 min.; androstanediol, 7.6-8.0 min.; T, 9.1-9.7 min.). The HPLC system consisted of a Waters Model 680 Gradient System equipped with a Hitachi Model 655α autosampler, Applied Biosystems Model 757 variable UV detector, and a Radiomatic Model A120 radioactivity analyzer. The conversion of T to DHT was monitored using the radioactivity flow detector by mixing the HPLC effluent with one volume of Flo Scint 1 (Radiomatic). Under the conditions described, the production of DHT was linear for at least 25 min. The only steroids observed with the human prostate and scalp preparations were T, DHT and androstanediol.

Inhibition studies

Compounds were dissolved in 100% ethanol. The compound to be tested was pre-incubated with the enzyme (either 5α-reductase type 1 or 2) prior to initiation by addition of substrate testosterone. IC_{50} values represent the concentration of inhibitor required to decrease enzyme conversion of testosterone to dihydrotestosterone by 50% of the control. IC_{50} values were determined using a 6 point titration where the concentration of the inhibitor was varied from 0.1 to 1000 nM.

A compound referred to herein as a 5α-reductase 2 inhibitor is a compound that shows inhibition of the 5α-reductase 2 isozyme in the above-described assay, having an IC_{50} value of about or under 100 nM.

A compound referred to herein as a 5α-reductase type 1 inhibitor is a compound that shows inhibition of the 5α-reductase type 1

isozyme in the above-described assay, having an IC_{50} value of about or under 100 nM.

A compound referred to herein as a dual 5α-reductase type 1 and 2 inhibitor is a compound that shows inhibition of both the type 1 and type 2 isozymes, having an IC_{50} value of about or under 100 nM for each isozyme.

EXAMPLE 3
ORAL COMPOSITION

As a specific embodiment of an oral composition of a compound of this invention, 5 mg finasteride and 40 mg simvastatin are formulated with sufficient finely divided lactose to provide a total amount of 580 to 590 mg to fill a size 0 hard gelatin capsule.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the casual variations, adaptations, modifications, deletions, or additions of procedures and protocols described herein, as come within the scope of the following claims and its equivalents.

WHAT IS CLAIMED IS:

1. A method of raising HDL cholesterol levels in a patient in need of such treatment comprising the administration of an HDL cholesterol raising dose of a 5α-reductase inhibitor.

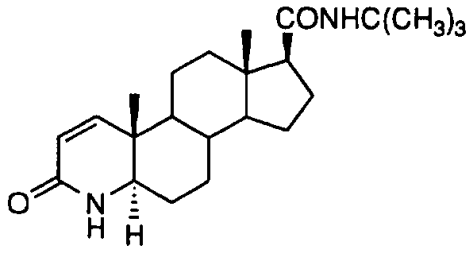
2. The method of raising HDL cholesterol levels of Claim 1 comprising the administration of one or more 5α-reductase inhibitors to the patient.

3. The method of Claim 2 wherein a Type 1 5α-reductase inhibitor and a Type 2 5α-reductase inhibitor are administered to the patient.

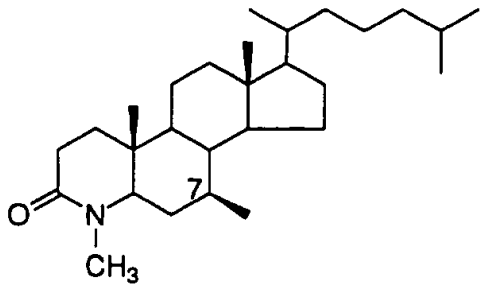
4. The method of Claim 1 wherein the 5α-reductase inhibitor is a Type 2 5α-reductase inhibitor.

5. The method of Claim 1 wherein the 5α-reductase inhibitor is selected from:

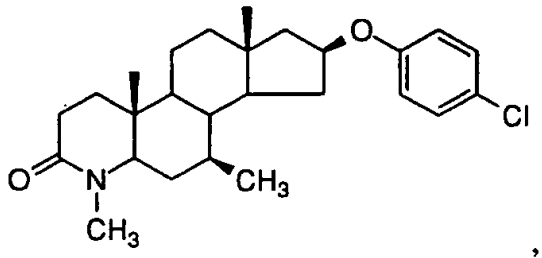
(1) finasteride



(2) 4,7β-dimethyl-4-aza-5α cholestan-3-one

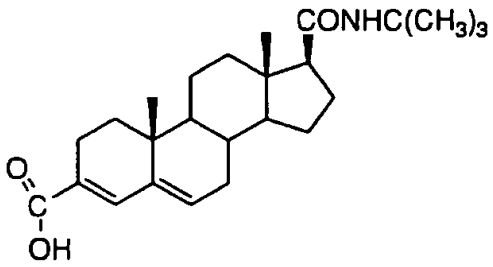


(3) 3-oxo-4-aza-4,7-β-dimethyl-16β-(4-chlorophenoxy)-5α-androstane

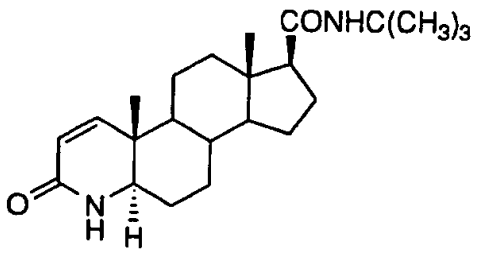


, and

(4) epristeride



6. The method of Claim 5 wherein the 5α-reductase inhibitor is finasteride:



7. The method of Claim 1 wherein the dose of the 5α-reductase inhibitor is from 0.2 to 1000 mg per day.

8. A method of reducing the risk of coronary artery disease mortality in a patient comprising the administration of an HDL cholesterol raising dose of a 5α-reductase inhibitor.

9. The method of reducing the risk of coronary artery disease of Claim 8 comprising the administration of one or more 5α-reductase inhibitors to the patient.

10. The method of reducing the risk of coronary artery disease of Claim 8 additionally comprising administration of a cholesterol-lowering agent selected from:

(a) an HMG-CoA reductase inhibitor,
(b) an HMG-CoA synthase inhibitor,
(c) a squalene epoxidase inhibitor,
(d) a squalene synthase inhibitor,
(e) probucol,
(f) clofibrate,
(g) niacin,
(h) fenofibrate,
(i) gemfibrizol, and
(j) a bile acid sequesterant.

11. The method of Claim 10 wherein the cholesterol lowering agent is an HMG-CoA reductase inhibitor selected from:

(a) lovastatin,

(b) simvastatin,

(c) pravastatin, and

(d) fluvastatin

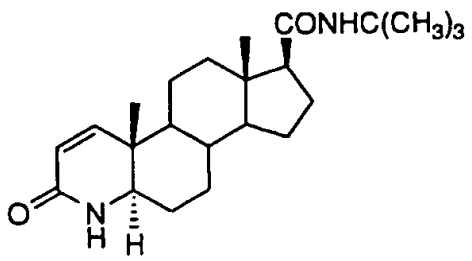
and pharmaceutically acceptable salts and esters thereof.

12. The method of reducing the risk of coronary artery disease of Claim 8 additionally comprising the administration of a pharmaceutically acceptable nontoxic cationic polymer capable of binding bile acids in a nonresorbable form in the gastro-intestinal tract.

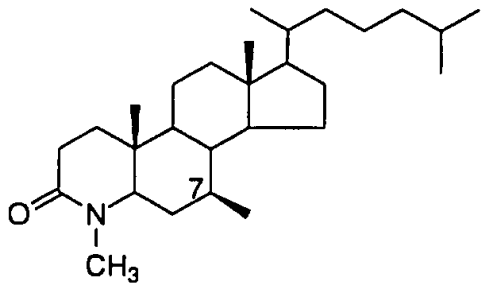
13. The method of Claim 12 wherein the pharmaceutically acceptable nontoxic cationic polymer capable of binding bile acids in a nonresorbable form is selected from cholestyramine, colestipol and poly[methyl-(3-trimethylaminopropyl)imino-trimethylene dihalide], and pharmaceutically acceptable salts and hydrates thereof.

14. The method of Claim 8 wherein the 5α-reductase inhibitor is selected from:

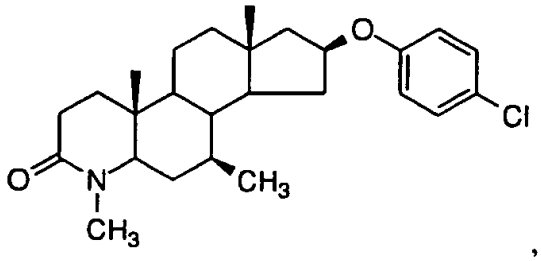
(1) finasteride



(2) 4,7β-dimethyl-4-aza-5α cholestan-3-one

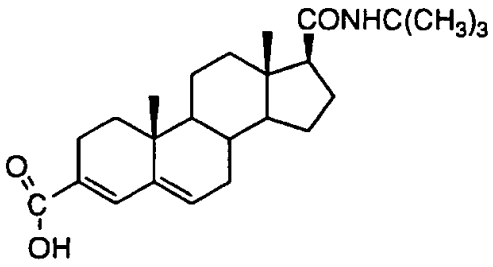


(3) 3-oxo-4-aza-4,7-β-dimethyl-16β-(4-chlorophenoxy)-5α-androstane

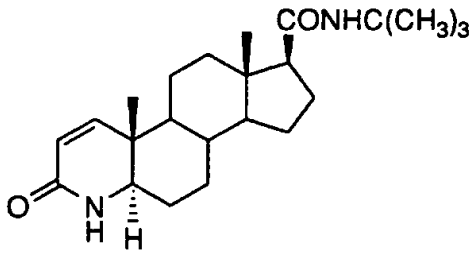


, and

(4) epristeride



15. The method of Claim 14 wherein the 5α-reductase inhibitor is finasteride:



16. A method of treating atherosclerosis in a patient in need of such treatment comprising the administration of an HDL cholesterol raising dose of a 5α-reductase inhibitor.

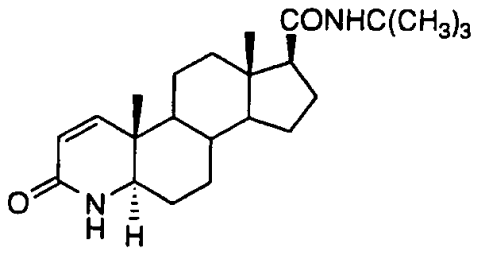
17. The method of treating atherosclerosis of Claim 16 comprising the administration of one or more 5α-reductase inhibitors to the patient.

18. The method of reducing the risk of coronary artery disease of Claim 16 additionally comprising administration of a cholesterol-lowering agent selected from:

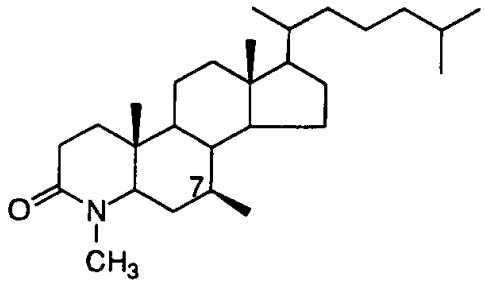
(a) an HMG-CoA reductase inhibitor,
(b) an HMG-CoA synthase inhibitor,
(c) a squalene epoxidase inhibitor,
(d) a squalene synthase inhibitor,
(e) probucol,
(f) clofibrate,
(g) niacin,
(h) fenofibrate,
(i) gemfibrizol,
(j) a bile acid sequesterant, and
(k) a pharmaceutically acceptable nontoxic cationic polymer capable of binding bile acids in a nonresorbable form in the gastro-intestinal tract.

19. The method of Claim 16 wherein the 5α-reductase inhibitor is selected from:

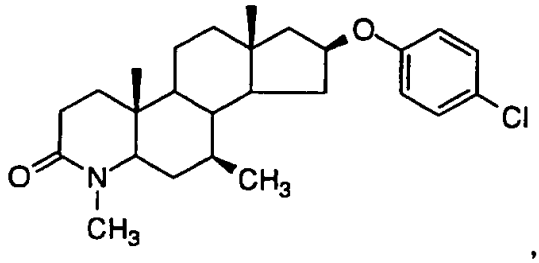
(1) finasteride



(2) 4,7β-dimethyl-4-aza-5α cholestan-3-one

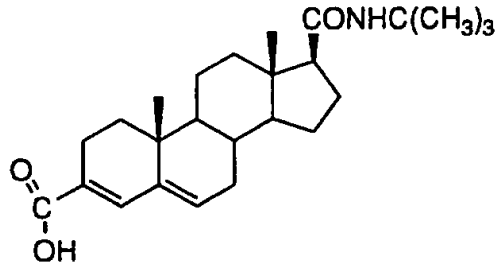


(3) 3-oxo-4-aza-4,7-β-dimethyl-16β-(4-chlorophenoxy)-5α-androstane



, and

(4) epristeride



20. A pharmaceutical composition comprising a 5α-reductase inhibitor, a cholesterol lowering agent selected from:

(a) an HMG-CoA reductase inhibitor,
(b) an HMG-CoA synthase inhibitor,
(c) a squalene epoxidase inhibitor,
(d) a squalene synthase inhibitor,
(e) probucol,
(f) clofibrate,
(g) niacin,
(h) fenofibrate,
(i) gemfibrizol,
(j) a bile acid sequesterant, and
(k) a pharmaceutically acceptable nontoxic cationic polymer capable of binding bile acids in a nonresorbable form in the gastro-intestinal tract;

and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

Inter ·nal Application No
PCI/US 95/07215

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/00 A61K31/58 A61K31/56

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO,A,95 00147 (MERCK & CO.) 5 January 1995 see page 1, paragraph 1 see page 2, line 21 - line 24 see claim 1 ---	1-19
A	PHARMACOMETRICS, vol. 47, no. 3, 1994 'Androgen Osaterone Acetate (TZP-4238)' see abstract see page 277 see page 281 --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 1996	07. 02. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Gerli, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J.CLIN.ENDOCRINOL.METAB., vol. 70, no. 4, 1990 pages 1136-41, 'Effects of finasteride (MK-906), a 5-alpha reductase inhibitor, on circulating androgens in male volunteers' see abstract ---	1-20
A	METABOLISM, vol. 39, no. 9, 1990 pages 919-24, 'HDL response to 5-alpha dihydrotestosterone and testosterone in Macaca Fascicularis: a hormone-responsive primate model for the study of atherosclerosis' see abstract -----	1-20

rnational application No.

PCT/US95/07215

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-19 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 95/07215

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9500147	05-01-95	AU-B- 7317794	17-01-95